

ART 34 AMDT

CLAIMS

1. A method of preparing a reprogrammed diploid cell which method includes
providing
5 a diploid donor cell or diploid donor nucleus, and
a recipient cell;
introducing the donor cell or donor nucleus into the recipient cell to produce
an aneuploid cell;
maintaining the aneuploid cell in a suitable environment for a period
10 sufficient to allow the donor nucleus to be reprogrammed;
optionally subjecting the aneuploid cell to an activation step; and
generating a reprogrammed diploid cell from said reprogrammed aneuploid
cell by substantial removal or substantial destruction of the recipient cell nucleus,
pronucleus, metaphase plate, chromatin, chromosomes or nuclear DNA from said
15 reprogrammed aneuploid cell.
2. A method according to Claim 1, wherein the recipient cell is an
oocyte, zygote, or embryonic blastomere.
3. A method according to Claim 2, wherein the oocyte is a metaphase
II oocyte.
- 20 4. A method according to Claim 1, wherein the recipient cell is an
embryonic stem cell, embryonic germ cell, primordial germ cell, embryonal
carcinoma cell or other pluripotent stem cell.
5. A method according to Claim 1, wherein the donor nucleus or cell is
a somatic cell or nucleus derived therefrom.
- 25 6. A method according to Claim 5, wherein the donor nucleus or cell is
a cumulus cell or nucleus derived therefrom.
7. A method according to Claim 1, wherein the donor nucleus or cell is

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an embryonic cell or nucleus derived therefrom.

8. A method according to Claim 1, wherein the donor nucleus or cell is a germ cell or nucleus derived therefrom.

5 9. A method according to Claim 1, wherein the donor nucleus or cell is an embryonic stem cell, embryonic germ cell, primordial germ or a somatic stem cell or nucleus derived therefrom.

10. A method according to Claim 1, wherein the donor nucleus or cell is transferred to the recipient cell by piezo-assisted micromanipulation.

10 11. A method according to Claim 1, wherein nucleus or nuclear DNA of the recipient cell is substantially removed or destroyed prior to division of the aneuploid cell.

12. A method according to Claim 1 wherein the donor cell is reprogrammed to an embryonic cell.

15 13. A method according to Claim 12 wherein the reprogrammed cell is capable of forming an animal embryo containing pluripotent embryonic cells.

14. A method according to Claim 13, wherein a pluripotent stem cell is derived from the pluripotent embryo cells.

15. A method according to Claim 14, wherein an embryonic stem cell line is produced.

20 16. A method of preparing a reprogrammed genetically modified diploid cell, said method including providing

a diploid donor cell or diploid donor nucleus which donor cell or nucleus has been genetically modified to eliminate or reduce an undesirable activity or to provide for or increase a desirable activity, and
25 a recipient cell;

introducing the donor cell or nucleus into the recipient cell to produce an aneuploid cell;

maintaining the aneuploid cell in a suitable environment for a period sufficient to allow the donor nucleus to be reprogrammed;

5 optionally subjecting the aneuploid cell to an activation step; and

generating a reprogrammed genetically modified diploid cell from said reprogrammed aneuploid cell by substantial removal or substantial destruction of the recipient cell nucleus, pronucleus, metaphase plate, chromatin, chromosomes or nuclear DNA from said reprogrammed aneuploid cell.

10 17. A method of preparing a reprogrammed genetically abnormal cell, said method including

providing

a diploid donor cell or diploid donor nucleus which donor cell or nucleus is derived from a genetically abnormal cell, such as a cell from an animal or person with a genetic disease, and

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a recipient cell;

introducing the donor cell or nucleus into the recipient cell to produce an aneuploid cell;

maintaining the aneuploid cell in a suitable environment for a period sufficient to allow the donor nucleus to be reprogrammed;

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optionally subjecting the aneuploid cell to an activation step; and

generating a reprogrammed genetically abnormal cell with an equivalent genetic composition to the said abnormal donor cell nucleus from said reprogrammed aneuploid cell by substantial removal or substantial destruction of the recipient cell nucleus, pronucleus, metaphase plate, chromatin, chromosomes or nuclear DNA from said reprogrammed aneuploid cell.

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18. A method of restoring or improving function of a tissue or organ, said method including

providing

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an animal, and

one or more reprogrammed cells according to Claim 1 or derivatives of said cells;

transferring the cells or derivatives thereof to the animal, preferably at or near the site of said tissue or organ; and

allowing the transferred cells or derivatives thereof to repopulate said tissue or organ.

- 5 19. A method of gene therapy, said method including providing
an animal, and
one or more genetically modified, reprogrammed cells according to
✓ Claim 16 or derivatives of said cells;
10 transferring the cells or derivatives thereof to the animal; and
allowing said cells or derivatives thereof to repopulate in said animal to
provide gene therapy.

20. A method according to Claim 1, further including the step of
generating a cell line, tissue, organ or animal embryo from said reprogrammed
15 cell.

21. A method according to Claim 20, further including the step of
generating a non-human animal from said animal embryo.

22. A method according to Claim 16, including the further step of
generating a genetically modified cell, cell line, tissue or organ or a transgenic
20 animal embryo from said reprogrammed cell.

23. A method according to Claim 22, further including the step of
generating a non-human transgenic animal from said animal embryo.

24. A cell produced by the method of Claim 1.

25. A genetically modified cell produced by the method of Claim 16.

- 25 26. A cell, cell line, tissue, organ or animal embryo produced by the
method of Claim 20.

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27. A genetically modified cell, cell line, tissue or organ or transgenic animal embryo produced by the method of Claim 22.

28. A non-human animal produced by the method of Claim 21.

29. A non-human transgenic animal produced by the method of Claim

5 23.

30. A method of generating an animal embryo which method includes providing

a diploid donor nucleus, and

a recipient cell; and

10 introducing the donor nucleus into to the recipient cell to produce an aneuploid cell;

optionally subjecting the aneuploid cell to an activation step;

15 generating a reprogrammed diploid cell from said aneuploid cell by substantial removal or substantial destruction of the recipient cell nucleus, pronucleus, metaphase plate, chromatin, chromosomes or nuclear DNA from said aneuploid cell; and

generating an animal embryo from said reprogrammed diploid cell.

20 31. A method of generating a transgenic animal embryo said method including

providing

a diploid donor nucleus which has been genetically modified to eliminate or reduce an undesirable activity or to provide for, or increase, a desirable activity, and

25 a recipient cell;

adding the donor nucleus to the recipient cell to produce a genetically modified aneuploid cell;

optionally subjecting the aneuploid cell to an activation step;

30 generating a reprogrammed diploid cell from said aneuploid cell by substantial removal or substantial destruction of the recipient cell nucleus,

pronucleus, metaphase plate, chromatin, chromosomes or nuclear DNA from said aneuploid cell; and

generating a transgenic animal embryo from said reprogrammed diploid cell.

5 32. A method of preparing an aneuploid or reprogrammed diploid cell which method includes

providing

a diploid donor nucleus,

an exogenous nucleic acid molecule, and

10 a recipient cell;

introducing the donor nucleus and the exogenous nucleic acid molecule into the recipient cell to produce an aneuploid cell; and

optionally generating a reprogrammed diploid cell from said aneuploid cell by substantial removal or substantial destruction of the recipient cell nucleus,
15 pronucleus, metaphase plate, chromatin, chromosomes or nuclear DNA.

33. A method of generating a transgenic animal embryo which method includes

providing

a diploid donor nucleus,

20 an exogenous nucleic acid molecule, and

a recipient cell;

introducing the donor nucleus and the exogenous nucleic acid molecule into the recipient cell to produce an aneuploid cell;

optionally generating a reprogrammed diploid cell from said aneuploid cell
25 by substantial removal or substantial destruction of the recipient cell nucleus, pronucleus, metaphase plate, chromatin, chromosomes or nuclear DNA; and

generating a transgenic animal embryo from the aneuploid or reprogrammed diploid cell.

34. A method of preparing a reprogrammed diploid embryonic cell or
30 embryo which method includes

providing

a diploid donor cell or diploid donor cell nucleus, and
a recipient oocyte or embryonic cell;

introducing the donor cell or donor cell nucleus into the recipient oocyte or
embryonic cell to produce an aneuploid cell;

5 maintaining the aneuploid cell in a suitable environment for a period
sufficient to allow the donor cell nucleus to be reprogrammed;

optionally subjecting the aneuploid cell to an activation step; and

generating a reprogrammed diploid embryonic cell or embryo from said
reprogrammed aneuploid cell by substantial removal or substantial destruction of
10 the recipient cell nucleus, pronucleus, metaphase plate, chromatin, chromosomes
or nuclear DNA from said reprogrammed aneuploid cell or one or more of its
daughter cells.

